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Six Days in Big Sky: Thermophiles 2011 (A Meeting Report)

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"*Thermophiles 2011*" held in Big Sky, Montana, USA during 11-16th September, 2011 was the eleventh international conference on thermophiles. More than 300 researchers from all corners of the globe discussed their research and findings on thermophiles viz. ecology, phylogenetics, evolution, physiology, metabolism, molecular and cell biology, genetic and environmental adaptations, mobile genetic elements, genomics and metagenomics of these fascinating high temperature loving microbes. The conference *Thermophiles 2011* was of twelve academic sessions.

Key words: Thermophiles, Big Sky, Montana.

Evolution, Ecology & Biogeochemical Processes

Miller's "primitive soup" hypothesis implying that the first living organisms used RNA as the information molecule has been challenged by the "metabolic life" theory. This hypothesis suggests that primordial "life" was nothing other than a series of self-catalyzed reactions based on simple compounds such as CO_2 and CO. Since many reactions are thermodynamically more favorable at high temperatures, it has been suggested that hydrothermal environments were the cradle of this "metabolic life." The enormous genomic diversity among hydrothermal ecosystems reflects the metabolic diversity permitted by the exceptional underlying geochemical diversity. The imprints of distinct geochemical environments can be found in the molecular compositions of microbial genomes and their protein products e.g transmembrane proteins of ancestral organisms were likely to be depleted in oxygen, paralleling the low oxygen content of Earth's atmosphere in the past (Acquisti *et al.,* 2007).

Everett Shock from Arizona State University demonstrated how geochemistry meets biochemistry in hydrothermal ecosystems. The thermodynamic energy costs of protein biosynthesis tuned to the chemical composition and energy supply determined by hot spring geochemistry. The major inorganic energy sources in the hydrothermal ecosystems of Yellowstone involves the oxidation of CO, hydrogen, magnetite, sulfur, hydrogen sulfide, methane and pyrite. He said that the microbial response to these energy options determine the structure of microbial community. Shock *et al.,* 2010 said that the geochemical compositions of water, gas, minerals and organic compounds in hydrothermal ecosystems permits thermodynamic analyses of the energy supplies that could be exploited by chemotrophic microbes and according to Dick and Shock, 2011 the protein compositions inferred from

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metagenomic data reside in local energy minima relative to the temperature and oxidation-reduction gradients in the hot spring outflow system. The greater Yellowstone ecosystem comprises the largest and most varied geothermal basin in the world. Cristina Takacs-Vesbach from University of New Mexico discussed a wide diversity of bacterial and archaeal populations of more than one hundred Yellowstone thermal springs including 15 new candidate phyla. The Phylogenetic and functional diversity have been detected by using a combination of conventional molecular biodiversity assays, metagenomic analysis and geochemical measurements. It has been suggested that pH is the major factor determining the composition of Yellowstone's thermal communities but dispersal and volcanism have also played a key role in the diversity of Yellowstone ecosystem. Tatyana Sokolova reported several new isolates from Guaymas Basin capable of hydrogenogenic growth on formate and established that the thermococci species are capable of sulfidogenic growth on CO or formate due to sulfur reduction.

Diversity and Ecology

Rachel Whitaker from University of Illinois who is known for her studies on the mechanisms of microbial speciation examining the high-resolution structure of diversity within and between microbial populations presented another dimension of microbial diversity with her current research on evolutionary dynamics from the chromosome to the community. Rachel Whitaker said that the sympatric speciation is driven by ecological specialization and gene flow in the form of recombination and horizontal gene transfer is restricted between incipient species and localized primarily to one region of the archaeal chromosome.

Sulfolobus islandicus is a thermophilic archaea that lives in biogeographically isolated populations in globally distributed geothermal regions, these isolated populations evolve in local environments and their genomes reflect adaptive changes selected by environmental conditions. Biogeography of the *Sulfolobus islandicus* pan-genome gave rise significant information on diversity and genome dynamics Michael L. Reno *et al.,* 2009. A current model of microbial genome dynamics partitions the variation into two distinct genomic components i.e. core and variable together called as the pan-genome. The core genome is composed of genes that are common to all strains and held stable through conservation. The variable genome is composed of genes not found in all strains, either because genes are gained through horizontal gene transfer or they are differentially lost. Every unique microbial strain sequence contains a different suite of variable genes. Yet, the ecological forces that define the distribution of these variable genes among strains the sources of genetic material and the rates at which genes are acquired and lost are unknown. It is largely believed that the variable gene component extends the physiological and ecological capabilities of microbial cells. Although the signatures from core housekeeping genes suggest that geographical, ecological and biological barriers promote divergence among the microbial populations. Allopatric speciation has been recognized in microorganisms using multilocus sequence analysis of conserved housekeeping genes. Jenny M. Blamey of Chile explained extreme microbiological diversity in Antarctica that is one extreme habitat where geographical isolation, long periods of darkness, great distances and extreme climate has imposed barriers to life. Despite its permanent frozen conditions, Antarctica contains a variety of environments including: hot springs, volcanic islands and deep-sea hydrothermal vents. The sum of these geologic, geographic and climatic characteristics has uniquely shaped the evolutionary history of prokaryotic life under extreme conditions. Phylogenetic studies based on the analysis of 16S rDNA gene suggest that many extremophilic microorganisms could represent remnant forms of primitive life that arose much earlier on our planet.

Very interestingly, Brian Hedlund, University of Nevada reported the isolation of a member of the GAL35 lineage which is a facultative microaerophilic heterotroph, dominant in Great Boiling Spring and represents a novel class of the *Chloroflexi*. Predicted genes in the draft genome encoding a putative carbon monoxide dehydrogenase (*coxMSL*), nitrite reductase (*nrfHA*) and nitrous oxide reductase (*nosZ*) suggest that this isolate may play important role in C and N cycling in GBS sediments. Through a combination of geochemistry, microbial cultivation and physiology, microbial community activity measurements and genomics an image of life in Great Boiling Spring (GBS), Nevada is beginning to come into focus. A unique aspect of life in Great Boiling Spring compared with Yellowstone springs of similar temperature is the importance of nitrification in energy, carbon, and nitrogen cycling. Scott Miller, University of Montana said that the ecological variation in thermal performance has evolved and maintained in the population despite the strong gene flow in most regions of the genome counter to the conventional wisdom that gene flow is absent during the process of divergence.

Scott Miller said that the combination of SNP genotyping and Illumina sequencing are used for identifying the genome that exhibit extreme genetic differentiation the progress in the biodiversity research is represented by viruses and their hosts in thermal hot springs. During the poster session Geoffrey S. Diemer (Portland State University) presented their investigations on virus diversity in Boiling Spring Lake (BSL): a fumarole heated acidic lake (pH 2.5, 55C-95C) located in Lassen Volcanic National Park, USA. Two complete Micro-virus genomes have been reconstructed from BSL viral metagenomic reads.

Genomics and Metagenomics

Metagenomic gene discovery provides an effective approach for accessing genomes and genes from the 'uncultured microbial majority' and offers realistic opportunities for accessing new areas of 'sequence space', 'function space' and even 'structure space'. Don A. Cowan, Institute for Microbial Biotechnology and Metagenomics University of the Western Cape suggested few

screening methods of thermophile metagenome expression libraries for the enzymes involved in the degradation of lignocellulosic substrates and he said that the expression and functional characterization of these gene products contributes to the long term objective of developing optimized enzyme cocktails for efficient saccharification of specific lignocellulosic feed stocks. Eugene V. Koonin explained the dominance of CRISPR-Cas adaptive immunity system in archaeal hyperthemophiles. He dwelt on the origin and evolution of the CRISPR-Cas systems after comprehensive analysis of the sequences and structures of Cas proteins by computational analysis and mathematical model development based on a cost benefit analysis of the CRISPR-Cas system in the course of its co-evolution with viromes of varying diversity. Todd Lowe, University of California spoke on new archaeal small RNAs and the evolution of genus-specific antisense RNAs which can act as regulators of transcription and translation.

Maria Mercedes Zambrano Colombian Center for Genomics and Bioinformatics of Extreme Environments – GeBiX, Carrera spoke on Metagenomics of microbial communities in hot springs of the Colombian Andes by using different culture-independent techniques 454 pyrotags, clone libraries and microarray. Matthew Stott, Wairakei Research Centre, New Zealand reported the genome sequence of *Chthonomonas calidirosea*, the first cultured representative of *Armatimonadetes*. The genome annotation confirms the ability to hydrolyse lignocellulosic compounds via the identification of a variety of cellulolytic, glycolytic and hemicellulolytic proteins.

Timothy McDermott,Thermal Biology Institute, Montana State University presented *Hydrogenobaculum* diversity in Yellowstone National Park and summarized about these bacteria in terms of *in situ* function, genomics and diversity. In the same session Mircea Podar spoke on functional genomics and evolution of nano archaea with special reference to interaction between the hyperthermophiles *Nanoarchaeum equitans* and *Ignicoccus hospitalis*. He compared the genome sequence of two additional species of *Ignicoccus* (*I. pacificus and I. islandicus*) and found them significantly larger than the genome of *I. hospitalis*, by which he gave a hypothesis that both *N. equitans* and its host co-evolved as well as streamlined their genomes. Anna-Louise Reysenbach Portland State University delivered a talk on how geology shapes microbial communities of hydrothermal vent deposits. She said deep-sea hydrothermal vents are important biogeochemical environments that support unique ecosystems rich in microbial diversity. The high temperature hydrothermal fluid mix with the cold oxygenated seawater, mineral precipitates to form vent deposits. These porous deposits get quickly colonized by a diversity of archaea and bacteria that harness the abundant geochemical energy available in the hydrothermal fluids.

Physiology & Metabolism

The genomic evidence for chemolithoautotrophy in high temperature archaeal communities and quantification of primary productivity in these high-temperature microbial communities was discussed by Ryan Jennings, Montana State University. He identified Crenarchaeota *Metallosphaera yellowstonensis* and other novel *Sulfolobales* are capable of autotrophic growth.

The physiological roles of the three [NiFe]-hydrogenase orthologs in the hyperthermophilic archaeon *Thermococcus kodakarensis* was among the highlights of the session. Tamotsu Kanai, Kyoto University, Japan talked about the function of three gene operons encoding [NiFe]-hydrogenase orthologs, Hyh, Mbh and Mbx. Hyh functions in the direction of H_2-uptake, Mbh responsible for H_2-evolution and Mbx in the S0 reduction process in *Thermococcus kodakarensis.* Arnold Driessen, University of Groningen, Netherlands discussed a novel class of antimicrobial proteins Sulfolobicins from Crenarchaeotes *Sulfolobus acidocaldarius* and *S. tokodaii.* Michael Adams,University of Georgia, spoke on the physiological roles and regulation of the three *Pyrococcus furiosus* hydrogenases. Hydrogenase I and hydrogenase II heterotetrameric enzymes located in the cytoplasm utilizes NADP (H) as an electron carrier. The III hydrogenase, termed MBH is an integral membrane protein complex encoded by a 14-gene operon. MBH functions as a novel respiratory system and both reduces protons to hydrogen gas and conserves energy in the form of an ion gradient that is used as ATP synthase for ATP synthesis.

Many marine hyperthermophiles accumulate solutes not only in response to osmotic stress but heat stress too. Most of these solutes have been rarely encountered in mesophiles (Santos *et al.,* 2011). The best example is di myo inositol phosphate (DIP), which has been found in members of almost all marine hyperthermophiles both bacteria and archaea and also in a few thermophiles. In general, the level of DIP increases remarkably at supra-optimal temperature, leading to the assumption that this solute plays a role in adaptation of hyperthermophiles to heat stress. Nuno Borges,Universidade Nova de Lisboa, Oeiras, Portugal explained about the role, evolution and regulation of the synthesis of di myo inositol phosphate (DIP) in hyperthermophiles. He said that DIP is part of the global strategy used by *Thermococcus kodakarensis* to cope with heat stress. Michael J. Danson,University of Bath explained about 2-oxoacid dehydrogenase multienzyme complexes (OADHCs) nature's largest multienzyme complexes of metabolism from the thermophilic archaea.

Frank T. Robb,University of Maryland presented findings on *Pyrococcus furiosus* archaeal chaperonin and its stabilization He reported that the alpha helix, 21GRDAQRMNILAARIVAETIR40, in the N terminal segment of Cpn-Pf was essential for the formation of the double-ring complex and that the double-ring structure was necessary for ATPase and chaperonin activities. Arg22 together with Glu37 from an adjacent helix constituted a stable ion pair and one such ion pair located at each end of the helix doublet locked the two helices, stabilizing the oligomer. John Spear,Colorado School of Mines, USA described about the microbial diversity of Yellowstone National Park stromatolite. Radiometric dating (^{228}Th/^{228}Ra, ^{228}Ra/^{226}Ra and ^{137}Cs) and in situ growth experiments reveal that the growth of a 5 cm stromatolite occurred on the order of years and light/dark laminae couples do not represent daily, weekly or seasonal cycles. Thus stromatolite growth rate and morphology, record the larger scale geologic processes in Yellowstone National Park.

Genetic Mechanisms

Horizontal or lateral gene transfer has been a major force for genome plasticity over evolutionary history and is largely responsible for the spread of fitness-enhancing traits including antibiotic resistance, virulence factors as well as for the adaptation of prokaryotes to extreme environments. Beate Averhoff,Goethe University Frankfurt/Main, Germany explained a tentative model of the unique transformation machinery of *Thermus thermophilus* HB27. She explained the unique transformation machinery and characterized two parts of it the secretin PilQ, a unique DNA-binding complex in the outer membrane that has a "cone" and "cup" structure plus five additional rings and the hexameric motor ATPase PilF.

The regulation of archaeal transcription fits more into the bacterial schema though there is close relationship of the basal transcriptional machinery between archaea and eukarya. Winfried Hausner (University of Regensburg, Germany) reported first time biochemical evidence for a transcriptional activator (PF1088) as a TFB recruitment factor in *Pyrococcus furiosus* archaea. The most interesting lecture perhaps in the session was from Xu Peng, University of Copenhagen who spoke on transcriptome analyses of *Sulfolobus islandicus* rudivirus 2 (SIRV2) and the infected host cells of *Sulfolobus solfataricus* using microarray and real-time PCR methods. About two thirds of the SIRV2 genes exhibited strong and temporal expression while the rest one third were weakly expressed. About 50 host genes were up-regulated and about 80 were down-regulated significantly.

Genetic and Environmental adaptations

The CRISPR-Cas system protects prokaryotes from viruses and other potential genome invaders (Caryn *et al.,* 2009). CRISPR-Cas systems are recently discovered, RNA-based immune systems that control invasions of viruses and plasmids in archaea and bacteria. Prokaryotes with CRISPR-Cas immune systems capture short invader sequences within the CRISPR loci in their genomes, and small RNAs produced from the CRISPR loci (CRISPR (cr)RNAs) guide Cas proteins to recognize and degrade the invading nucleic acids. John van der Oost ,Wageningen University, Netherlands explained the prokaryotic antiviral defense mediated by CRISPR RNA. Malcolm White,University of St Andrews, Scotland explained the characterization of CASCADE and CMR complexes in the hyperthemophile *Sulfolobus solfataricus.*

Tamar Barkay,Rutgers University, US explained about thermophilic origin of the mercury resistance (mer) system and its evolution which is constrained by the unique conditions of heat impacted ecosystems. The ability to sustain microbial life in presence of the toxic metal mercury (Hg) depends on the reduction of ionic to volatile elemental Hg by the coordinated activities of Hg transporters and a mercuric reductase (MerA). Hg resistant microbes may have a critical role in modulating life in these ecosystems by converting aqueous phase to volatile elemental Hg. Sonja-Verena Albers,Max Planck Institute for terrestrial Microbiology, Germany delivered a talk on role of different surface structures in crenarchaeal biofilm formation by constructing single deletion, double deletion and finally the triple deletion mutants of any cell surface structure. These mutants were tested in surface attachment assays as well as for biofilm formation. Interestingly, the flagella only played a minor role in these processes whereas the aap pili were important for adhesion and the UV induced pili for the interaction of the cells within a biofilm.

Mobile genetic elements

Aurore Gorlas spoke on a novel virus TPV1 (*Thermococcus prieurii* Virus 1) discovered in a hyperthermophilic euryarchaeote isolated from a deep-sea hydrothermal chimney sample collected at a depth of 2700 m. Till date only one virus particle (PAV1) was isolated from a hyperthermophilic euryarchaeon *Thermococcales* in addition to TPV1 both these viruses have similar morphologies (lemon-shaped) but share only two homologous ORFs hence he proposed that PAV1 and TPV1 should be included in the same novel viral family but to different viral genera. Marie Gaudin reported various virus-like vesicles with unique features (including long flexible filaments and strings of vesicle) from deep-sea hydrothermal vent *Thermococcales* species (euryarchaeota). She explained about the mechanism of vesicle production and their protein in three strains of Thermococcales: *Thermococcus kodakaraensis, Thermococcus gammatolerans* and *Thermococous* sp. 5-4 using different electron microscopic techniques and analyzing their major protein composition. The rod-shaped virus SIRV2 of *Sulfolobus islandicus* and the icosahedral virus STIV of *S. solfataricus* employ an extraordi-nary virion release system, involving the formation of Virus-Associated Pyramids (VAPs) on the host cell membrane (Brumfield *et al.,* 2009; Bize *et al.,* 2009). Tessa Quax, Institute of Pasteur, France reported that the VAPs consist of multiple copies of SIRV2-coded 10 kDa protein P98. She told that this protein is capable to self-assemble into pyramidal structures and form VAPs when expressed in the hyperthermophilic acidophilic archaeon *S. acidocaldarius* and even in the mesophilic bacterium *Esch-erichia coli.*

Applications

David Mead (Lucigen Corp., Middleton WI) spoke on new tools for functional analysis of genes and metagenomes. He explained about a linear "pJAZZ" vector for E. coli expression which can holds large inserts (up to 30 kb) of DNA. Its low copy number (2-4/cell) is ideal for high stability protein production. He emphasized that hundreds of new carbohydrate metabolizing-enzymes (CAZymes), thermostable DNA polymerases can be captured and expressed from dozens of genomes and metagenomes by using this system. Guangyu Yang from Shanghai Jiao Tong University explained that the combination of protein and solvent engineering may provide an effective approach to improve activities of promiscuous enzymes, which may be of great interest to be used to create natural-rare hyperthermophilic enzymes. T. Satyanarayana , University of Delhi , India shared his findings on applications of a thermostable and alkalistable xylanase. His group has isolated a gene encoding xylanase of 1.077 kb from environmental metagenomes which is optimally active at 80 ÚC and pH 9.0. The enzyme being alkalistable and thermostable can find application in pre-bleaching of paper pulps for mitigating the chlorine requirement in pulp bleaching and thus in developing an environment-friendly pulp bleaching process. Amy M. Grunden (North Carolina State University, Raleigh, NC, USA) stated that archaeal superoxide reductase (SOR) provides a novel mechanism to reduce superoxide and archaeal genes can be used to alter plant metabolism. The transgenic expression of *Pyrococcus furiosus* SOR in plants systems provides the plants protection against environmental stresses.

Nitrile hydrolyzing enzymes possess widespread applications right from bioremediation, biocatalysis to pharmaceuticals. The use of nitrile hydrolyzing enzymes in the preparation of optically pure carboxylic acids and amides is of great biotechnological potential.The enantio- and regio-selective attribute of these enzymes again plays a massive role in the bio-transformations (Banerjee *et al.,* 2002). Rajesh K. Sani, South Dakota School of Mines and Technology, Rapid City, SD isolated several thermophilic cellulose and xylan-degrading pure cultures belonging to the genera *Brevibacillus, Paenibacillus, Bacillus*, and *Geobacillus*. These thermophiles grew on various inexpensive regional carbon and energy sources (e.g., prairie cordgrass, corn stover) and produced ethanol from microcrystalline cellulose in a single step. He said that these thermostable enzymes and robust thermophilic fermentative microbes will facilitate development of more efficient and cost-effective forms of the simultaneous saccharification and fermentation process to convert lignocellulosic biomass into biofuels.

Virus

Kenneth Stedman,Portland State University, USA delivered a talk on the virus component of an unique ecosystem Boiling Springs Lake which is an acidic (~pH 2), hot (50-95 ^{o}C) lake in Lassen Volcanic National Park. The vast majority of the BSL metavirome sequences (>96%) were not detectably similar (by MG-RAST analysis) to sequences in public databases. He reported less than 0.5% of the sequences were similar to known virus sequences. Thomas Schoenfeld,Lucigen Corporation, Middleton, WI explained about PyroPhage Pol. the first thermostable viral DNA polymerase and among the first Pols of any source with thermo stability and reverse transcriptase (RT) activity for efficient single-enzyme RT-PCR. He said that this enzyme is the basis for the recently introduced PyroScript RT-PCR Master Mix and a single-enzyme isothermal RT-LAMP amplification system useful for point of care detection of infectious RNA viruses. This enzyme has inherent high fidelity which facilitates its use in preparative PCR. Masatada Tamakosh and his team fromTokyo University of Pharmacy and Life Sciences shared their findings on *Thermus thermophilus* phage fTMA having similar morphology and genome length with fYS40 with a difference of broader host cell specificity.

The last session of Thermophiles 2011 was dedicated to a great researcher of Thermal biology research for his long and significant contribution to the knowledge and was named as Juergen Wiegel session. Juergen Wiegel (University of Georgia Athens) spoke on the spatial variation of microorganisms in two different hot springs, Arkashin Shurf and Zarvarzin II located in the Uzon Caldera in Kamchatka (Far East Russia). He elucidated possible correlation between the genetic divergence and the spatial and geochemical properties of the springs by quantifying the intra-species heterogeneity using the 16S rRNA and eight housekeeping genes and he concluded that the spatial variation is influenced by both spatial separation and geochemical properties of these hot springs. Frank F. Roberto and Mark Young from Biological Systems Department, Idaho National Laboratory, USA, and Thermal Biology Institute, Montana State University, USA reported novel archaea from thermoacidic springs of Yellowstone National Park. They concluded that Metagenomic studies close the gap between cultivated and uncultivated species by revealing sequences that may truly encode metabolic capabilities. Jennifer Littlechild ,Exeter Biocatalysis Centre, University of Exeter, UK delivered a talk on thermozymes structural determination and commercial applications. She said that structural determination of many of these thermozymes provides information regarding mechanisms of protein thermo-stabilisation, enzymatic mechanistic and substrate specificity. The Exeter Bio-catalysis Centre developed many naturally stable enzymes found in thermophilic microorganisms. She presented on many enzymes L-aminoacylase from *Thermococcus litoralis,* gamma lactamase from *Sulfolobus solfataricus* and alcohol dehydrogenase from *Aeropyrum pernix* developed at Exeter already used commercially and enzymes in development include a transaminase (Sayer 2009), a dehalogenase (Rye *et al.,* 2009) and a carboxylesterase from *Sulfolobus* species.

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